Development and Regeneration of Podostemon Ceratophyllum

A Dissertation

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

BAYARD L. HAMMOND

JUNE, 1935

Reprinted from THE BULLETIN OF THE TORREY BOTANICAL CLUB, Vol. 64, pages 17-36, and THE BOTANICAL GAZETTE, Vol. 97, pages 834-845.

Development and Regeneration of Podostemon Ceratophyllum

A Dissertation

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

BY

BAYARD L. HAMMOND

JUNE, 1935

Reprinted from THE BULLETIN OF THE TORREY BOTANICAL CLUB, Vol. 64, pages 17-36, and THE BOTANICAL GAZETTE, Vol. 97, pages 834-845.

R. UNIV.
BIBLIOTHEEK
LEIDEN

Separately printed, without change of paging from Bulletin of the Torrey Botanical Club 64: 17-36. January, 1937.

Development of Podostemon ceratophyllum[1]

Bayard L. Hammond

(with 36 figures)

Warming (1881, 1882) published two papers in Danish with a French résumé, dealing with the vegetative and reproductive organs of *Podostemon ceratophyllum* Michx. Although these studies are rather complete, especially with regard to external morphology, further observations were needed on the development of this species. No record has been found of any observations of this species since Warming's publications. Willis (1902) treated related species and summarized the literature to date. Went (1909, 1926) worked on a number of species and included many important references to the literature. The family was treated in some detail by Engler (1930), who cited most of the important literature.

The living plants were observed in the field over a period of several years at Beaver Dam Creek near Cockeysville, Maryland, at Gwynn's Falls near Baltimore, Maryland, at Difficult Run on the Virginia side of the Potomac River near the Great Falls, and at Cabin John Creek near Washington, D. C. Material for laboratory study was collected at each of these places.

HABITAT AND HABIT

Podostemon ceratophyllum (fig. 1) is a creeping, alga-like, fresh water dicotyledon native to the eastern region of North America from New Brunswick, west to Ontario and Minnesota, and south to Georgia and Mississippi. The plants are found on rocky or gravelly bottoms in streams, usually where the water is rapidly moving and evidently well aerated. They are usually found in the shallow, sunlit parts of the rapids. They have, however, been observed to grow to a depth of about three feet where the water is swiftly flowing. The roots are small (1 millimeter or less in diameter). They are photosynthetic and plagiotropic, creeping about over the substratum in all directions. Phototropically they are not very sensitive and are often found growing underneath stones. They usually grow attached to rocks and pebbles of the substratum by root hairs and by peculiar fleshy disk-like structures, the haptera. Occasionally they may be found growing on bits of wood or any other objects wedged in between the rocks. Where sand collects among the plants, the roots may adhere to pebbles and often form dense tangles throughout the sand. The color of these plants ranges from an olive green to a dark reddish brown depending on the exposure to light. Plants exposed to more intense light are usually

[1] Botanical contribution from the Johns Hopkins University, no. 142.

darker in color. The reddish brown color, common in the family, is due to the presence of anthocyan in the surface cells. The parts of plants found under stones range from green to light yellow. If the yellow parts are exposed, they soon attain the usual reddish brown color.

Podostemon ceratophyllum behaves as an herbaceous perennial. When winter approaches, the more delicate parts of the plant die back, leaving only the older parts of the roots and the basal portions of the stems to winter over. During the first few warm days of early spring, vegetative activity is quickly resumed. This is of two sorts: first, the formation of branch roots, and second, the regeneration of the apices of the old stems and roots. The precise manner in which regeneration takes place has been described in an earlier paper by the author (1936).

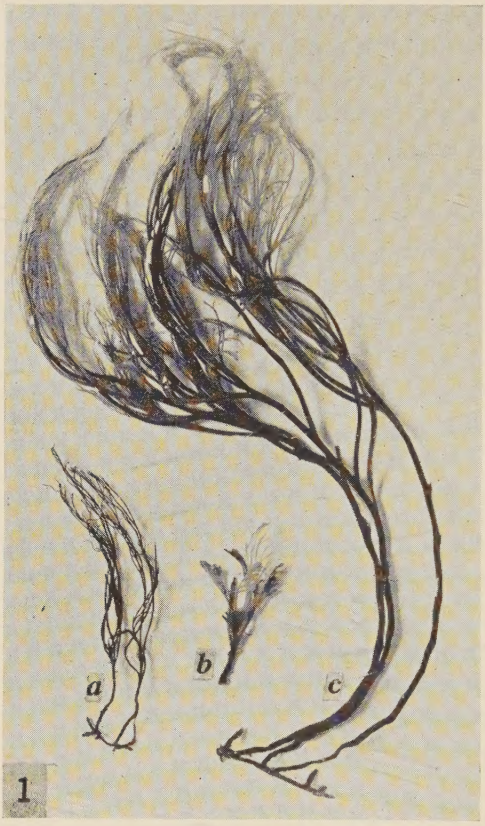


Fig. 1. Three forms of *Podostemon ceratophyllum* found in the vicinity of Baltimore and Washington. All were collected in the early part of August, 1933. $\times\frac{1}{2}$.

The flower buds are initiated sometime in May and push out from the leaf bases in early June. They never open while the plants are submerged although occasionally the peduncles of those near the surface of the water may elongate considerably. Unopened buds have been observed as late as November. However, when the buds are exposed by the lowering of the water level, they soon rupture their spathellas by the elongation of the peduncles. The latter elongate to about 5 millimeters or more. During this time the filaments of the stamens also elongate considerably. The capsules ripen within a few days, during which time the delicate leaves quickly wither and the erect stems and peduncles gradually become dry. The exact method of pollination was not ascertained. Since the flowers are not noticeably fragrant, and are some-

what inconspicuous, it seems likely that they are either anemophilous or autogamous.

It is evident that the Podostemonaceae are best adapted to regions where there are marked wet and dry seasons, such regions as exist in many parts of the tropics, where the majority of the species of the family grow. Along the Atlantic coast of North America there are no well marked wet and dry seasons and flowering apparently depends on the occurrence of a dry summer or at least on a marked temporary drop of the water level at the proper flowering season. At Difficult Run, during the summer of 1932, the level of the water lowered considerably, and the plants flowered abundantly, but in 1933 the plants remained submerged the entire season and not a single flower was seen. Propagation by seeds is thus evidently dependent on the occurrence of a dry period during the summer, but vegetative propagation is very effective and easily compensates for the lack of seed production except for wide dispersal.

It has been noticed repeatedly that during the growing season the plants are subject to periods of dying back, such as that which normally occurs during the winter, i.e., leaves, together with the apical portions of the stems and the delicate apices of the roots, disappear. This condition seems to be associated with periods of high temperature, such as that which occurred during the unusually hot summer of 1931. In the early part of July of that year the plants began to die back and remained in a very unhealthy state until October, when vigorous vegetative activity was resumed. This condition occurred simultaneously in all the colonies under observation.

Owing to their preference for swiftly moving water, the plants are unusually free from competition by other aquatic plants, few of which can persist there. At Gwynn's Falls there were found intermingled with them occasional plants of a species of *Potamogeton*, which, however, did not seem to offer much competition. In the colony at Difficult Run the small red alga, *Lemanea*, was commonly present on the rocks, but because of its small size it undoubtedly was insignificant in competition with the quickly growing roots of *Podostemon*. Various forms of insect larvae, especially those of the Stone-fly (*Perla* sp.) and Dobson-fly (*Corydalis cornuta*), were commonly found among the plants, but their presence, however, apparently had little effect on them.

Several forms of *Podostemon ceratophyllum* were found. The plants at Gwynn's Falls and those of Beaver Dam Creek are of medium size and apparently are of the same variety (fig. 1a). At Difficult Run, however, several varieties grow side by side in colonies of various sizes, and the plants of a given colony are usually uniform in size and structure, although

gradations between them seem to exist in some cases. One of the smaller forms (fig. 1b) has stems from about 1 to 1.5 centimeters long, bearing broad, flat, ribbon-like leaves. A larger form (fig. 1c) collected at the same time, is very coarse, with stems attaining a length of 20 centimeters or more, and the leaves are long and finely divided. The roots of this form are on the average slightly larger than those of the ordinary form. In order to determine whether or not the difference in form is due to differences in the rate of the flow of water or in the amount of aeration, transplantations to other streams were undertaken. In the summer of 1933, plants of the two extreme forms were tied to pieces of galvanized iron wire screen. Some were placed alongside of those growing at Gwynn's Falls and others were planted in a stream near by where *Podostemon* had not been found. The plants were observed for several weeks, during which time they continued to grow well and retained their original characteristics.

During the course of this investigation the writer received from Professor S. H. Derickson of Lebanon Valley College some preserved specimens of *P. ceratophyllum* collected by him near Harrisburg, Pa. These plants are of a small variety and are different in general appearance from any of those observed near Baltimore and Washington, D. C.

DEVELOPMENT AND STRUCTURE OF THE VEGETATIVE AND REPRODUCTIVE ORGANS

The seedling. The germination of the seed of *Podostemon ceratophyllum* has not been observed.[2] The mode of germination of the seed and the early development of the seedling in many tropical genera of the family, including species of *Podostemon*, is, according to Willis (1902), uniform throughout. It is, therefore, probable that *P. ceratophyllum* may prove to show the same type of germination. In *P. subulatus*, according to Willis (1902), the hypocotyl emerges as the water rises, bends downwards, and attaches itself to the substratum by root-hair-like rhizoids. Its base soon becomes expanded, or tuber-like, thus forming a larger surface for attachment by rhizoids. The seedling is now nearly upright; the cotyledons expand, several leaves appear in two ranks, and the further growth of the primary axis ceases. In this, as well as in all other investigated species of the family, there is no developed primary root. The endogenously formed secondary root emerges from the base of the hypocotyl, usually at right angles to the plane of the cotyledons.

[2] Seeds assumed to be mature were placed on wet filter paper but these always failed to germinate. It is quite probable that they were sterile, for material from the same place, which was sectioned and examined for the study of the development of the embryo sac, showed no embryos. Other evidences of sterility will be pointed out in the discussion of the development of the embryo sac.

The mature root. The root of *Podostemon ceratophyllum* is dorsiventral and is usually slightly flattened, especially on the ventral side. The form of the roots varies from nearly cylindrical to strongly flattened. It is provided with a one-sided, i.e., dorsiventral, root cap, in which the side toward the substratum is less developed than the opposite side. Root hairs are present on the ventral side of the root. These apparently secrete an adhesive substance, which assists in attaching the plant to the substratum. There is also present on the root another structure, the hapteron of Warming (1881), which is the chief organ of attachment of the plant (h, fig. 4).

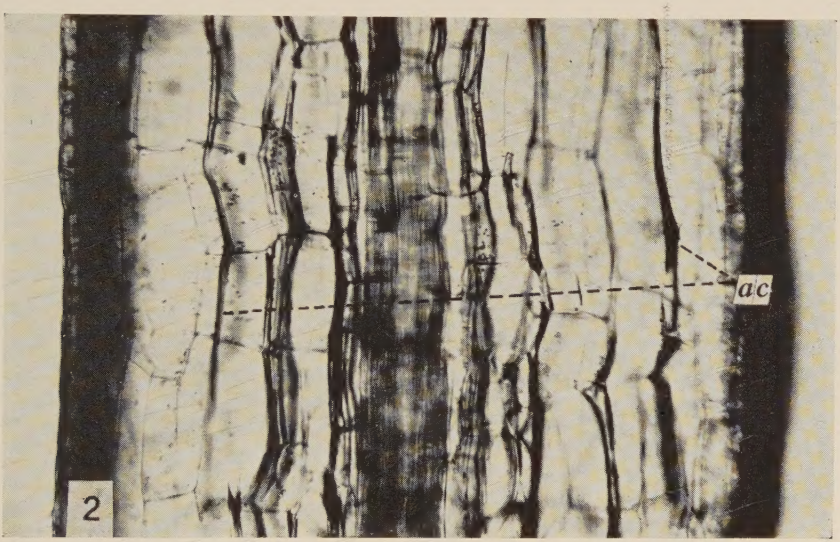


Fig. 2. Longitudinal hand-cut section of the petiole of a fresh leaf, showing air canals (ac). ×70.

It appears on the ventral side of the root directly under the base of each shoot. The writer has found them also on the ventral side near the base of a lateral root, and Warming found haptera developing also on the shoots. These structures arise exogenously from the surface of the root and each has a terminal growing point. They are parenchymatous. The writer could find no true vascular tissue in them. They appear merely as extensions of the cortical tissue, probably stimulated to growth in some manner by the initiation of the growing points of the shoots. The haptera are very sensitive to both gravity and contact, and they soon become firmly attached to the substratum by an adhesive secretion. In doing so, they form a broad disk at the surface of the substratum, or at other times they may become much branched, forming finger-like structures even before the substratum is reached. Warming (1881) believed the haptera to be derived phylo-

genetically from the root because of their apical growth, their position on the roots and, their capacity for possessing root hairs, which latter he observed in only one case. Went after many years study of this family also reached this opinion. In his recent study of *Mourera fluviatilis* (1926a), however, he suggests that the haptera can not be metamorphosed roots "because they are certainly exogenous, whereas roots in Podostemonaceae are endogenous, just as with all other plants." Went also suggests that, since they are sensitive to contact, they may be compared with tendrils, "only with the difference that they develop in water."

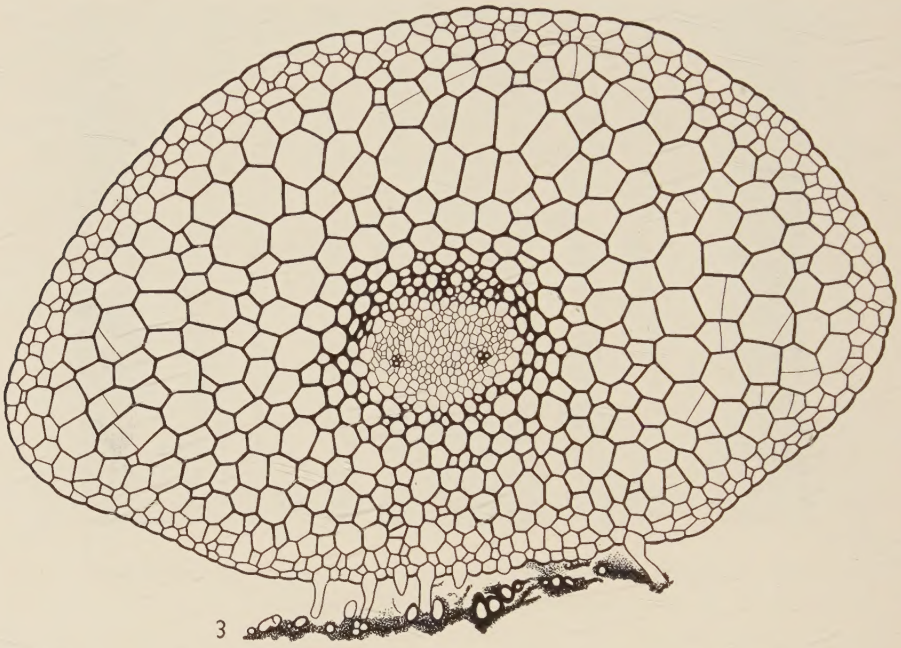


Fig. 3. Transverse section of mature root, showing two vascular bundles near the center and root hairs below, partially embedded in an adhesive substance.

Concerning the structure of the root, Warming (1881) showed: (1) the central cylinder is nearer the ventral side and contains two strands of xylem with phloem above each, (2) companion cells could not be demonstrated beside the sieve tubes, (3) the inner cortical cells are strongly collenchymatous and often pitted, (4) no definite endodermis is differentiated about the central cylinder, and (5) chloroplasts and silica granules are abundant in the outer cortical cells and epidermis.

To the above the writer can add the following observations. The root hairs, which are present on more or less irregular patches on the lower

surface of the root, serve as organs of secretion and attachment. They arise from the epidermal cells of the root, and, before reaching the substratum, many of them branch often rather complicatedly (fig. 5). The root hairs are apparently always uninucleate and their nuclei are much larger than those of the cortical cells or those of the ordinary epidermal cells. After reaching the substratum, the hairs grow along the surface usually parallel with the axis of the root (fig. 3). The horizontal portions of adjoining hairs become compactly grouped, secrete the adhesive substance, and soon their walls become greatly thickened (fig. 3). The vertical portions of the hairs, however, remain more or less thin-walled. Obviously these structures do not function as absorbing organs as in most other plants.

The dorsiventrality of the root is shown clearly (fig. 3) by the position of the central cylinder. As the root there shown is fully mature and the cortical walls have become thickened, the fundamental tissue of the central cylinder is clearly differentiated from the surrounding tissues by its smaller, and in all cases seen, thin-walled cells. The older cells of the entire cortex on the contrary tend to become collenchymatous. Pitted walls are common in such thickened cells and may occur throughout the entire cortical tissue. The median cortical cells of the dorsal region of the root are very large, while the cells of the first and second sub-epidermal layers are much smaller. Partition walls, usually periclinal, are often found in the cortical cells of old roots (fig. 3).

Each of the two xylem strands of the central cylinder mentioned by Warming is made up of from one to six or eight spiral or annular tracheids (fig. 3), which are small in diameter, have pointed ends and are often very long. Occasionally a few tracheids may be seen scattered between these two groups. Nowhere in the material studied have true vessels been found. The phloem, which is situated above the xylem groups, is very poorly differentiated. A cambium, even a fascicular cambium, is absent and as a result there is no secondary xylem or secondary phloem.

The outer walls of the epidermal cells may become heavily cutinized, especially in the furrows between these. Cutinization also occurs on the anticlinal walls of the epidermal cells and often may occur on the inner walls. This cutinization may extend inward for two or three layers of subepidermal cells. Upon injury, the cells thus exposed may become cutinized at the surface.

Origin and development of the lateral root. No primary root has been reported in any species of the Podostemonaceae. This seems likely to prove true also of *Podostemon ceratophyllum*. Hence, all roots in plants of this family are either adventive, i.e., from the hypocotyl, or from later seg-

ments of stems (see Hammond (1936)), or are lateral branches of such adventive roots. Every branch root of *P. ceratophyllum* is always of the same type and assumes the same functions as the parent root from which it arises. As there are no sharply defined tissue layers in the root, it is impossible to demonstrate exactly the place of origin of the lateral root in relation to an endodermis or of the tissue layer of the parent root which gives rise to the "root pocket." (The term "root pocket" is used here to designate the protective structure which is first developed and comparable in origin to that found in certain aquatics such as *Lemna*, *Eichornia*, etc.) The "root pocket" is derived, as will be presently shown, from one of the layers of larger cells which apparently belongs to the cortex. If the root be considered a reduced structure, it is possible that in this reduction the endodermal layer has become dedifferentiated, i.e., certain structures such as Casparian strips or other such thickenings which are usually present in this layer in allied plants, have disappeared from it in *Podostemon*.

The parenchyma of the central cylinder (fig. 3) is composed of small, thin-walled, and elongated cells. The cortex, as has already been noted, is composed of relatively large, and nearly isodiametric cells, whose walls usually become somewhat thickened with age and often pitted. Between these two regions the transition in the size of cells, as seen in transverse section, may be more or less gradual. In longitudinal sections there is evident a rather abrupt boundary between the elongated cells of the central cylinder and the large, nearly isodiametric cells of the cortex. If the inner cortical cells become strongly collenchymatous, the transition between thin-walled and thick-walled cells is quite abrupt.

The growing point of the lateral root is initiated within the small cells of the central cylinder in a region external and lateral to either bundle. The first evidence of such a growth is seen in the tangential divisions of one of the layers of cells not far from the small group of tracheids of one of the vascular bundles (fig. 6). This layer is more or less obscurely differ-

Figs. 4–10.*—Fig. 4, sketch of portion of plant. ×3. Fig. 5, sketch of unattached root hairs in various stages of branching. ×80. Fig. 6, portion of transverse section of a main root, showing bundle and origin of lateral meristem. ×93. Fig. 7, transverse section of root, showing meristem of a branch root. ×93. Fig. 8, longitudinal section of root through meristem of branch root, showing root-cap layer before outer cortical tissue is broken down. ×93. Fig. 9, longitudinal section of main root, showing a more advanced stage in formation of lateral root. The cortical tissue has begun to break down and the initiation of the root cap is clearly seen. ×93. Fig. 10, transverse section of parent root, showing a late stage in the development of the lateral root. The root-cap layer on the ventral side has first broken loose from the other cells of its layer. ×93.

* Abbreviations: *h*, hapteron; *l*, leaf; *lr*, lateral root; *r*, root; *rcl*, root-cap layer; *rh*, root hairs; *sh*, shoot; *st*, stipule.

rcl
lr
9
lr
rcl
8
l
st
rh
sh
h
r
4
7
5
tr
6
rcl
lr
10

entiated around the cylinder and the initiation of the lateral root meristem beside a bundle occurs in such a way that the direction of growth of the lateral root is away from the bundle and at right angles to the plane joining its xylem and phloem. A tangential section would show the initial meristematic region to be strongly oblong, owing to the fact that tangential divisions progress more quickly along the length of the central cylinder in both directions from the point of origin of the meristem than in the direction perpendicular to this. On the contrary, a tangential section of the lateral root meristem in most vascular plants is circular or nearly so. By transverse divisions and by still more tangential ones, there is soon formed in this initial region a group of meristematic cells with very dense cytoplasm and large nuclei (figs. 7, 8). The meristematic region continues to enlarge by radial growth and tangential cell divisions. These occur more rapidly in the center, and soon an apical zone is formed which is covered by the dermatogen, and in which the cells now divide in all planes (fig. 9). The cells back of this zone now divide more often by walls transverse to the young lateral root and then there arises in the latter a zone of elongation (fig. 10), followed by the differentiation of the central cylinder. The mode of origin of the lateral root just described is that always found in the material studied, and, contrary to views generally accepted as to the origin of the lateral root in this family as a whole, it develops in this species with a *definite* relation to the central cylinder.

Soon after the group of meristematic cells is formed, the cells of the layer covering this region begin to divide by transverse and tangential walls (figs. 8, 9, 10). This layer, which gives rise to the "root pocket," appears usually to be the inner layer of nearly isodiametric cells of the cortex. Such cases show that the innermost layer of the cortex is probably equivalent, in this respect at least, to an endodermis. But as the layer is not characterized by the usual markings of a normal endodermis, it can not be definitely identified with this. The cells of the layer usually show only a few divisions while the young lateral root is still within the tissue of the parent root. This layer, for the most part, retains its original peripheral dimensions until the young root is about to emerge. For this reason it is not difficult to trace the development of the "root pocket" from its very early stages, particularly as the boundary walls of the layer are thicker than the walls either of the cells formed within the layer or of those of the meristematic region underneath (figs. 8, 9).

As the young lateral root continues to develop, the tissues external to the layer which gives rise to the "root pocket" become stretched and are soon broken down (figs. 9, 10). The root forces its way out, largely by mechanical pressure. At some stage before the young root has emerged,

the layer just referred to becomes ruptured in a circle a short distance back of its apex. This rupture, however, takes place first on the ventral side, a fact which accounts for the dorsiventral form of the "root pocket" (fig. 10). If we examine a section of a root showing the lateral root after the "root pocket" has become free, it is difficult to determine the origin of the latter, since its cell layer near the proximal end, where the meristem was initiated, often divides further and becomes indistinguishable from the adjacent cortical tissue. As the lateral root emerges, the cells of the "root pocket" often continue to divide until the latter may become as many as several cells in thickness. The "root pocket" rarely develops further. It is soon replaced, however, by the root cap proper, which is formed from the meristematic tissue underneath the "root pocket." The root cap is also dorsiventral from a very early stage. The exposed portion of the lateral root soon expands to approximately the diameter of the root which gave rise to it, principally by the enlargement of the cells of the cortical tissue.

As in other vascular plants, there is no actual union of the tracheids in the primary xylem of the lateral root with those of the main root. Since no secondary xylem is formed in *Podostemon*, there is no provision for the conduction of water from lateral root into main root through tracheids. That such a connection may be easily dispensed with in these plants is due to the fact that the lateral root, as soon as it emerges, is independent. The differentiation of the tracheids of the lateral root begins at the base of the lateral root usually quite near the bundle of the main root. The first few tracheids formed are often very large in diameter and short compared with those formed later.

From the preceding discussion it is clear that a root has only two rows of laterals, all members of which retain the dorsiventral symmetry of the parent root, regardless of the position of the latter. The lateral roots emerge often in considerable numbers, commonly several side by side.

The shoot. Warming (1881) has already given a detailed description of the shoot. He shows in brief that the shoots are usually paired (fig. 4) and arise acropetally in the lateral cortical tissue just back of the growing point of the root. These shoots grow out transversely to the root and more or less erectly from the substratum. The leaves are alternate, in two ranks, and the plane passing through the two ranks also passes through the axis of the root. There is no stem growing point. The leaves arise each from the base of the second older leaf, i.e., from the next older leaf in its rank. (The details of origin and development of leaves and shoot were discussed in an earlier paper by the writer (1936).) Each leaf has a complete intrapetiolar stipule, i.e., the two stipules are grown together across the adaxial face of

their own leaf (fig. 4). The lateral branches of the shoot are not axillary within these ordinary stipules but arise at the base of the abaxial side of the leaf (the "bord notoscope" of Warming) and are covered by a stipule-like structure (intrapetiolar stipule) formed on this same side of the leaf stalk, i.e., on the side opposite the ordinary stipules. For such leaves provided with two intrapetiolar stipules, Warming proposed the term "feuilles dithéques." Thus there are formed two dichotomous branches and the dithecous leaf is then found in the center of the dichotomy.

The blade of the mature leaf is ordinarily divided or forked into twenty to thirty or more linear lobes, each lobe containing an axial bundle of small elongated cells. The tissue surrounding the bundle is composed of large parenchymatous cells containing chlorophyll. Chlorophyll is present in the epidermis of the leaf as well as in that of the stem and root. Stomata are entirely absent from all parts of this plant as they are in all members of the family. It has commonly been stated that intercellular air spaces are quite absent from the leaves of the Podostemonaceae. This is not true in the case of the large form of *Podostemon ceratophyllum* found at Difficult Run, the leaves of which regularly have definite intercellular air channels running lengthwise through the cortical tissue (fig. 2).

The mature leaf contains no xylem elements (tracheids). The present writer, however, has found that a very young leaf does show tracheids in its bundle. But, as growth proceeds, the formation of xylem elements does not keep pace with the growth of the leaf. As a result, any transverse section, except at the base of the petiole, would fail to show any xylem elements, and here they are often collapsed, owing to an inward pressure on them of the rapidly growing parenchyma cells.

The flower. Warming (1881) has described in detail the structure of the flower. The flower is usually terminal on the branch and is enclosed at first by the adaxial pair of fused stipules. The initiation of the flower is accompanied by a branching of the stem. The branch arises at the base of the abaxial side of the same leaf and is enclosed at first by its own pair of abaxial fused stipules. The flower is solitary, naked, and zygomorphic, with its plane of symmetry corresponding to that of the stem. The bud is enclosed before flowering in an oval capsule-like structure, the spathella. The stamens, the filaments of which are fused at their bases for more than half their length, are borne on the ventral side of the flower where the stalk joins the ovary. Each anther contains four sporangia. Two staminodes arise, one on each side of the pair of stamens, and a scale is present at the notch between the two filaments. Warming reported several instances in which three stamens developed instead of two. The writer has found many flowers of this type, also several in which only one stamen had developed.

Figs. 11–24.*—Fig. 11, sagittal section of an ovule, showing an initial cell of the outer integument. ×285. Fig. 12, sagittal section of an ovule, showing enlargement of an epidermal cell, which is to give rise to an initial of the inner integument. ×285. Fig. 13, sagittal section of ovule, in which there has been a periclinal division of the epidermal cell and in which the outer cell is in process of cutting out an initial of the inner integument. ×285. Fig. 14, a similar section through ovule after the initial of the inner integument is formed. ×285. Fig. 15, longitudinal section of ovule, showing that the initial, which is to give rise to the inner integument, may be either the chalazal or the micropylar half of the epidermal cell. ×285. Fig. 16, transverse section through the nucellus and terminal cell of its axial row, showing two cells of the epidermis, which have divided by periclinal walls. ×285. Fig. 17, sagittal section of ovule, showing archesporial cell and relation between outer and inner integuments. ×285. Fig. 18, longitudinal section of nucellus, showing two axial archesporial cells. ×285. Fig. 19, longitudinal section of ovule, showing four archesporial cells, probably a later development of an ovule similar to that shown in figure 18. ×285. Fig. 20, longitudinal section through inner integument and nucellus. Here the two asymmetrically placed archesporial cells and the two axial cells below them are apparently equivalent to the four archesporial cells shown in figure 19. ×285. Fig. 21, longitudinal section of the nucellus in which only one of the terminal axial cells has enlarged to form an archesporial cell. ×285. Fig. 22, longitudinal section of ovule, showing three archesporial cells. ×285. Fig. 23, longitudinal section of ovule, showing four archesporial cells. The cell in dotted outline is from the adjacent section. ×285. Fig. 24, longitudinal section through abnormal ovule, showing relation between integuments, the stalk between these, and direction of growth of the ovule.

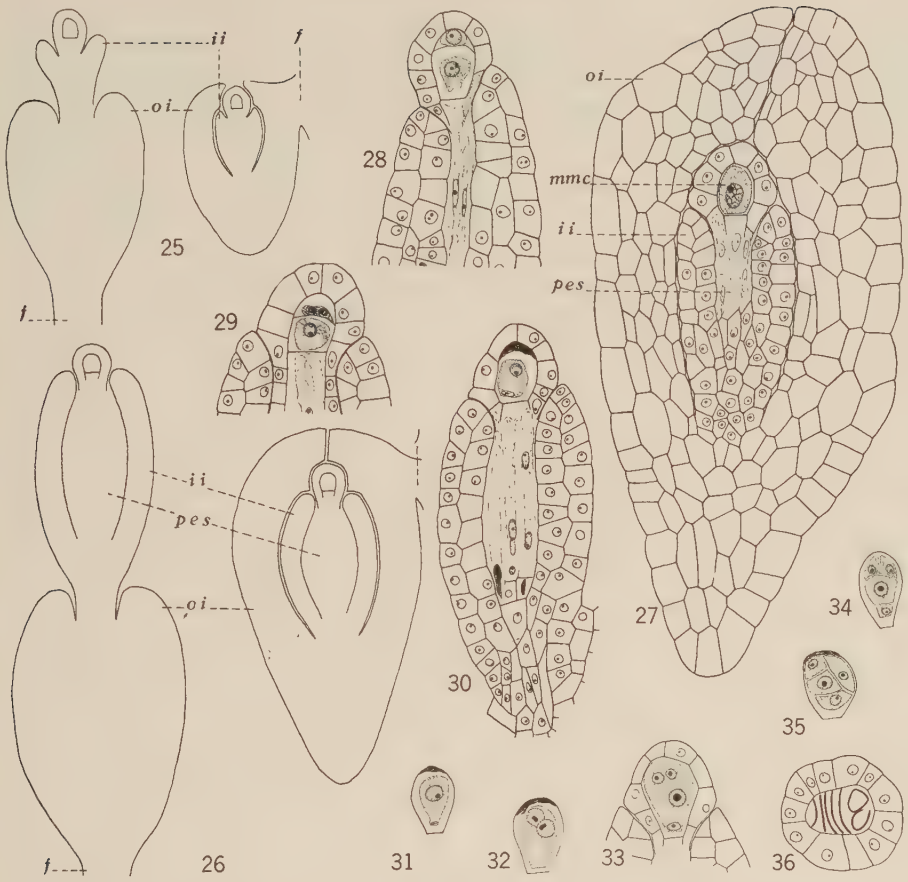
* Abbreviations: *f*, funiculus; *ii*, inner integument; *oi*, outer integument.

The ovary is two-celled and provided with two nearly sessile stigmas, curved slightly toward the stamens. Although two is the usual number of stigmas, occasionally the writer found ovaries provided with three. The placenta is axile, and bears from half a dozen to forty or more anatropous ovules.

The ovule and archesporial cell. The ovule arises on the axile placenta as a protuberance initiated by the growth and periclinal divisions of one or a few subepidermal cells with the accompanying growth and anticlinal divisions of the epidermal cells overlying these. The young ovule at a slightly later stage consists of one or more axial rows of cells derived from the periblem, surrounded by an epidermis. As the development of the young ovule progresses, the protuberance becomes differentiated into a funicular and a nucellar region, and at about this time a bending of the funiculus takes place by which the ovule ultimately becomes anatropous. At the time of appearance of the outer integument the axis of the nucellar portion lies nearly at right angles to the base of the funiculus. In the ovules of *Mniopsis Weddelliana* Tul., as described by Warming (1882) and in those of other members of this family described by Went (1909), the funiculus bends earlier and the nucellus soon assumes a position nearly parallel with the base of the funiculus.

The outer integument makes its appearance before the inner one. Warming's figures (1882) of the formation of the outer integument of *Mniopsis* do not show definite initials. Went (1909) in his studies of several species of the family describes it as "simply aris[ing] as an annular fold on the nucellus." In *Podostemon ceratophyllum*, however, the integument usually arises from a definite ring of wedge-shaped initials, each initial being formed from an epidermal cell by means of an oblique anticline (fig. 11). At an angle of from 60 to 70 degrees to the oblique wall of the first initial cell a second wall cuts out a new initial. By the continuation of this process and by the accompanying periclinal divisions of the epidermal cells derived from the initials the outer integument is formed. The mature integument is usually several cells in thickness (fig. 27).

After the outer integument has attained a length of several cells, the inner integument is initiated also by a ring of wedge-shaped initials. These initials arise as follows: A ring of epidermal cells near the basal portion of the nucellus above the place of origin of the outer integument begins to enlarge (fig. 12). Occasionally this enlargement is preceded by a periclinal division of the epidermal cell, dividing it into a peripheral and a basal cell, the initial being cut from the peripheral cell (figs. 13, 14). At this stage an obliquely transverse anticline divides each cell, and, just as in the case of the outer integument, *either* the *chalazal or* the *micropylar* cell of the



Figs. 25–36.*—Fig. 25, a schematic diagram, comparing an abnormal ovule with a normal one both at the archesporial-celled stage. Detailed explanation in text. Fig. 26, a schematic diagram of a similar pair of ovules after the integuments and pseudo-embryo sac are fully formed. Detailed explanation in text. Fig. 27, longitudinal section of ovule, showing megaspore-mother cell, relation of integuments, and formation of pseudo-embryo sac. ×285. Fig. 28, longitudinal section of embryo sac before the terminal cell has degenerated. ×285. Fig. 29, longitudinal section of embryo sac after the terminal cell has divided previous to degeneration. ×285. Fig. 30, longitudinal section of embryo sac, pseudo-embryo sac, and inner integuments. The basal cell has divided to two by a spindle longitudinal to the embryo sac. Note the smaller size of the lower nucleus. ×285. Fig. 31, similar section of embryo sac shown in figure 30 in which the lower nucleus has almost completely degenerated. ×285. Fig. 32, longitudinal section of embryo sac after the functional megaspore has divided to two. ×285. Fig. 33, longitudinal section of embryo sac after the functional megaspore has undergone two successive divisions. ×285. Fig. 34, longitudinal section of mature embryo sac. Cleavage of the cytoplasm has taken place, forming two synergids above, an egg cell at the center, and a polar nucleus below. ×285. Fig. 35, similar section of mature embryo sac shown in figure 34 in which the two synergids are arranged in a different manner in relation to egg cell. ×285. Fig. 36, section, not quite transverse, of embryo sac in which the contents have disappeared, and its wall has become spirally thickened. ×285.

* Abbreviations: *f*, funiculus; *ii*, inner integument; *mmc*, megaspore-mother cell; *oi*, outer integument; *pes*, pseudo-embryo sac.

resultant two remains as an epidermal cell while the other, a wedge-shaped initial, develops to form the inner integument (fig. 15). Went (1909), however, in his study of the inner integument of several tropical forms states that only "the upper [micropylar] half remains an epidermal cell of the nucellus, while the lower [chalazal] half develops to form the inner integument." Segment walls now continue to appear in such a manner that the resulting inner integument consists of two layers, an inner and an outer one (fig. 17). Occasionally in contrast to the cases studied by Went the inner integument may become three layered as a result of tangential divisions in one of the two original layers (fig. 28). Although tangential walls are infrequent, radial walls and others transverse to the ovule regularly occur.

At this stage the apical portion of the nucellus above the place of origin of the integuments consists of a single axial row of cells surrounded by a layer of epidermal cells. Occasionally there may occur tangential divisions in the latter (fig. 16), and sometimes the nucellus may consist of more than one axial row of cells, often resulting in the formation of more than one archesporial cell. From an early stage in the development of the nucellus, the terminal (sub-epidermal) cell of the central cell row remains undivided, while the basal ones continue to divide. At some period after the formation of the integuments and before the outer integument has entirely enclosed the nucellus, this terminal cell, the primary archesporial cell, becomes differentiated, this being indicated by its large size, dense cytoplasmic contents, and large nucleus. Not uncommonly two sister archesporial cells are formed (fig. 18). It is clear that these arise by a longitudinal division of the terminal sub-epidermal cell.

Most members of this family have but one archesporial cell in each nucellus. The only exception reported up to the present time is in *Mourera fluviatilis*, where in one instance Went (1926b) found two archesporial cells. This is a rather common phenomenon in the material of *Podostemon ceratophyllum* used in this study, in which not only one or two archesporial cells may occur but more rarely as many as three or four (compare figs. 18–23). The arrangement of the several archesporial cells may differ in different cases.

Aside from the fact that the outer integument develops before the inner, an unusual sequence among the angiosperms, but constant throughout this family, another very interesting developmental phenomenon is found here. The inner integument at first remains small and compact about the base of the nucellus, while the outer integument by rapid growth entirely encloses the inner integument and the nucellus. Thus the outer integument only forms the long narrow canal, the micropyle, at the apex

of the ovule (fig. 27). As the ovule continues to develop, the cells of the nucellus near the base of the inner integument fail to divide rapidly enough to keep pace with the elongation of the integuments, and these cells are consequently drawn out into shred-like structures, later leaving a cavity, the "pseudo-embryo sac," surrounded by the inner integument, and into which, according to Went and others, in all the species studied by them, the developing embryo grows (figs. 27, 30). The apical portion of the nucellus, consisting of the terminal axial cell, which is ultimately to become transformed into the embryo sac, surrounded by one layer of epidermal cells, is thus carried forward at the narrow mouth of the elongating inner integument, and this terminal portion remains between the endostomium and exostomium (fig. 27).

Certain abnormal ovules of the type illustrated in figure 24 give a possible explanation of the presence of the pseudo-embryo sac in this family. For the purpose of comparing the normal and the abnormal ovule, figure 25 will be considered. This is a schematic diagram at the same magnification of the two types of ovules, both at the archesporial-cell stage. The first striking difference here is seen in their size and shape. The abnormal ovule in this case is, as usual, straight while the normal one is of the anatropous type. The second point of difference is that the axial tissue between the inner and the outer integuments of the abnormal ovule elongates to form a stalk (fig. 24). At this stage, the inner integument of an abnormal ovule is sometimes more massive than that of the normal one, and may often consist of three instead of the ordinary two layers.

Figure 26 compares the two types of ovules at a later stage in which the integuments are fully formed. The chief points of interest here are that in the abnormal type the outer integument, instead of continuing to grow up about the inner integument and nucellus, develops to form a bulky, aril-like mass of tissue at the base of the stalk, and that in both cases a pseudo-embryo sac is present. It is also noted that the inner integument is much more bulky in the abnormal form than in the normal one.

If the abnormal development of these ovules should be disregarded, it might be concluded, as it sometimes is, that the pseudo-embryo sac is a result of the pressure of the outer integument, which develops before the inner and holds the inner integument tightly against the nucellus, thus separating the apical portion of the nucellus from the region at the base of the elongating inner integument. When, however, we consider the advanced stage of the abnormal ovule just described, we must conclude that the pseudo-embryo sac is formed without the aid of the outer integument. In reality it is the inner integument that fails to enlarge at the endo-

stomium and thus clasps the nucellus, which, upon the elongation of the integument, is ruptured at this point.

Other types of abnormality are quite frequent, such as irregularly formed integuments, and the protrusion of the nucellus out of both integuments into the cavity of the ovary. Many instances have been observed in which no ovules are formed. In such cases, the placenta usually becomes quite fleshy.

The embryo sac. The writer has experienced some difficulty in obtaining material to demonstrate the normal development of the embryo sac. Material collected at Difficult Run, Beaver Dam Creek and Gwynn's Falls showed various types of abnormality and, until material was secured from Cabin John Creek in July 1935, the evidently normal course of development shown by this material was not discovered.

The development of the embryo sac is striking in many respects. There are no tapetal cells. The megaspore mother cell (fig. 27) divides by a transverse wall into two cells (fig. 28). The upper cell becomes aborted but its nucleus may occasionally divide again before its degeneration takes place (fig. 29). The remaining nucleus divides to two by a spindle longitudinal to the embryo sac (fig. 30). The lower nucleus of the two so formed is smaller and soon degenerates (figs. 30, 31). The remaining nucleus undergoes two successive divisions (figs. 32, 33), and then a cleavage of the cytoplasm takes place, thus forming two synergids, one egg cell and one polar nucleus (figs. 34, 35). Except for occasional irregularities the development of the embryo sac in this material corresponds rather closely with that described by Went (1926a) in all the tropical members of the family he studied. Went states that endosperm is absent in all species of this family and the writer has failed to find any in *Podostemon ceratophyllum.*

The origin of the abnormal embryo sacs observed in the material mentioned above can not be explained satisfactorily. In these the early development agrees with that just described for normal sacs, but what takes place at later stages is very uncertain. Embryo sacs of opened flowers that would normally be mature may contain but one large, single nucleus or two or three ordinary sized ones Occasionally many small nuclei are present. This latter condition may be due to a fragmentation in nuclear division as an irregular distribution of chromosomes has been observed in several cases. Such irregularity has also been seen in the formation of the microspores, in some of which as many as six or eight small nuclei are not infrequently found. In some embryo sacs a cleavage of the cytoplasm may take place with only two or three nuclei present. The cause of such deviations may prove to be attributable to hybridization, an explanation suggested by the various forms of this plant mentioned previously.

In this study several abnormal cases have been observed in which the contents of the embryo sac, after the time of pollination, have disappeared. The wall of the embryo sac becomes lignified with spiral thickenings like those of a tracheid (fig. 36). Whether this condition is a result of age and disintegration of the unfertilized embryo sac is undetermined.

SUMMARY

Podostemon ceratophyllum is an herbaceous perennial, which renews its growth in the spring by the formation of new endogenous branch roots and by very active regeneration from all parts of weathered-off roots and shoots.

No seedlings were seen. From the standpoint of reproduction by seeds, *P. ceratophyllum* in its areas of distribution does not meet with a type of climate so well suited to the general family habit, of flowering only when exposed during a dry period in the growing season, as do most tropical Podostemonaceae. Vegetative activity throughout the growing season is usually extremely luxuriant as long as the plants are submerged. In some summers, however, the plants are subject to periods of dying back, owing apparently to prolonged spells of high water temperature.

The "root pocket" of the lateral root is formed from a cortical layer of cells of the parent root. It is, however, not a permanent structure, being later replaced by the root cap proper. Both the "root pocket" and root cap are dorsiventral and the origin of this symmetry can be seen in the early development of the lateral root.

The root hairs are often very much branched, and they appear to be highly specialized as organs of secretion and attachment.

A mature leaf shows xylem elements only at the base of its petiole.

The ovary normally bears from half a dozen to forty or more ovules, but ovaries with no ovules are not infrequent. The integuments of the ovule arise from rings of wedge-shaped initials, formed by anticlinal walls in the epidermal cells. Depending on the direction of the oblique walls, either the chalazal or the micropylar halves of the original epidermal cells of the ring become the initials of the integuments.

Usually one archesporial cell is formed in each ovule, but occasionally two, three, or four, side by side or one above the other, have been observed. The mature embryo sac is somewhat pear-shaped and usually contains two synergids, one egg cell and one polar nucleus. The embryo sac in the greater part of the material studied shows a marked irregularity in its development. Certain abnormal ovules show clearly that the pseudo-embryo sac is formed by the constriction of the endostomium about the base of the nucellus at the time of elongation of the inner integument.

Occasionally the contents of an unfertilized embryo sac disappear and its wall becomes spirally thickened like that of a tracheid.

This investigation was undertaken at the suggestion of Professor Duncan S. Johnson, and it is a pleasure to acknowledge his helpful advice, interest, and encouragement.

THE JOHNS HOPKINS UNIVERSITY
BALTIMORE, MD.

Literature cited

Engler, A., and Prantl, K. 1930. Natürlichen Pflanzenfamilien. **18a**: 1–68.

Hammond, B. L. 1936. Regeneration of *Podostemon ceratophyllum* Michx. Bot. Gaz. **97**: 834–845.

Warming, E. 1881. Familien Podostemaceae. Kongel. Dansk. Videnskab. Selskabs Skrifter. Sjette Raekke. **1**: 1–34.

————. 1882. Familien Podostemaceae. Kongel. Dansk. Videnskab. Selskabs Skrifter. Sjette Raekke. **2**: 77–130.

Went, F. A. F. C. 1909. The development of the ovule, embryo sac, and egg in Podostemaceae. Rec. trav. bot. Néerl. **5**: 1–16.

————. 1926a. Morphological and histological peculiarities of the Podostemonaceae. Proc. Intern. Cong. Plant Sciences. Ithaca. **1**: 351–358.

————. 1926b. Untersuchungen über Podostemaceae III. Verh. k. Akad. Wetensch. Amsterdam, 2. Sect., **25**, No. 1: 1–58.

Willis, J. C. 1902. Studies in the morphology and ecology of the Podostemaceae of Ceylon and India. Ann. Roy. Bot. Gard. Peradeniya. **1**: 267–465.

Reprinted for private circulation from
THE BOTANICAL GAZETTE, Vol. XCVII, No. 4, June, 1936
PRINTED IN THE U.S.A.

BAYARD L. HAMMOND

REGENERATION OF PODOSTEMON CERATOPHYLLUM[1]

BAYARD L. HAMMOND

(WITH NINETEEN FIGURES)

Introduction

Probably no family of the angiosperms exhibits greater capacity for regeneration than do the Podostemonaceae. This capacity has become evident during a study undertaken to ascertain to what extent regeneration occurs in each of the vegetative organs of *Podostemon ceratophyllum* Michx. WARMING (2) figured several roots which had evidently regenerated their tips, and also a hapteron (organ of attachment) which had regenerated its tip; but since he used preserved material, he was apparently unable to trace the exact origin of these regenerated structures. The present study has shown that this process may occur not only in the root but also in the stem and leaf.

Podostemon ceratophyllum (figs. 2, 4) is an alga-like, aquatic dicotyledon found on rocky or gravelly bottoms of rapids in streams of North America east of the Mississippi River. Its roots are plagiotropic, dorsiventral in structure, and chlorophyll-bearing. The shoots are also dorsiventral in structure and they arise acropetally from the root, usually in pairs a short distance back of the root growing point. The roots attach themselves to the substratum by root hairs and by fleshy disk-like or finger-like haptera. The latter arise exogenously from the ventral side of the root, each under the base of a shoot (fig. 2).

The anatomy of the plant differs in several respects from that ordinarily exhibited by dicotyledonous plants. In the root the central cylinder lies nearer the ventral side, and is composed of two xylem strands, lying side by side, with poorly developed phloem above each. There is no differentiated endodermis, and the primary meristem layers are therefore not clearly defined. The same is also true of

[1] Botanical contribution from the Johns Hopkins University, no. 132.

the stem, in which its two or three bundles are not arranged in a cylinder but in a crescent-shaped row. Each leaf possesses in its petiole a single bundle of elongated cells. For further information, see Hammond (1).

Investigation

Culture methods

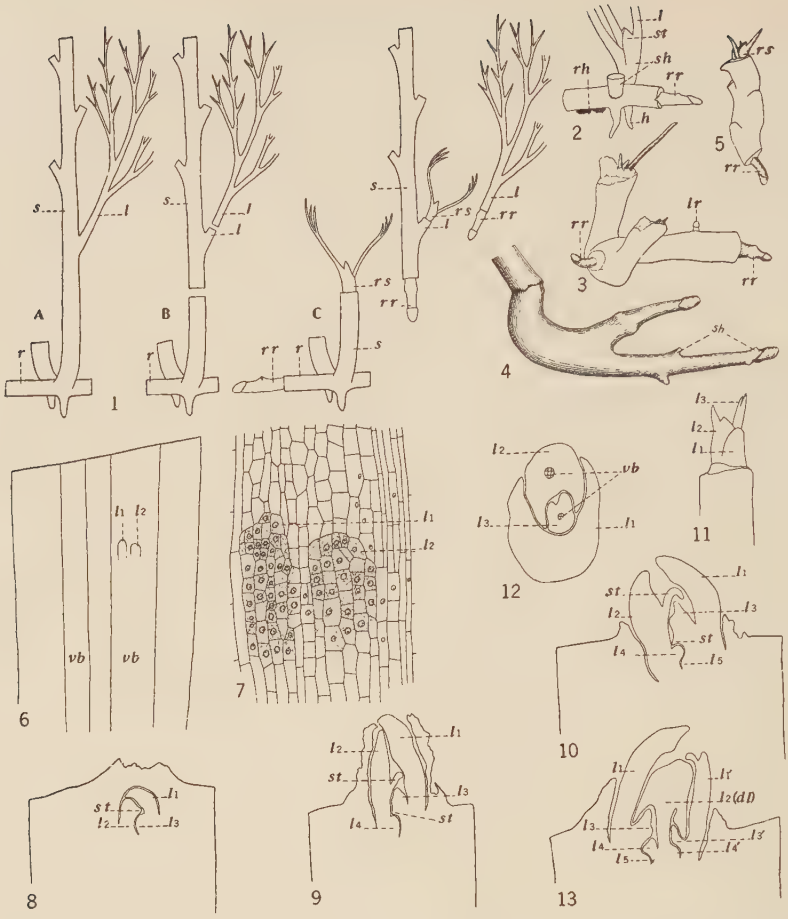
The plants seem to require constantly and rapidly moving, well aerated water, and to the lack of this may largely be attributed their failure to thrive in laboratory culture. Went (3) reports complete inability to grow any species artificially. As it was found desirable to grow parts of the plant in the laboratory, the following four methods were tried, with the problem of aeration kept in mind.

1. Stones on which the plants were growing were placed in a jar of water through which a stream of air bubbles was supplied from an aerating system. This system is one often employed in work of this type, using the discharge from an aspirator to force air into a jar of water. Although the plants showed signs of growth, various algae soon collected in the culture and this method was consequently discontinued.

2. Pieces of stem and root were placed on wet filter paper in petri dishes, where they were kept moist and had free access to the gases of the air. Although many of the fragments regenerated and continued to grow for more than a month, the regenerated parts were weakly developed and this method was not very satisfactory, although it is convenient for studying early stages.

3. Plants grown in the greenhouse were placed in wire-screen trays under a fine spray of tap water. Here even pieces of the leaves regenerated. The regenerated parts began to appear in about four days on all organs and became about as robust as they do in their natural environment. New root growing points continued to develop on the regenerated roots indefinitely, but the leaves of the shoots from such roots always died back in a few weeks.

4. The most effective method was that of placing the plants, in wire-screen trays provided with covers, back in the stream by the undisturbed plants, when they grew just as did the latter. Because of the inconvenient distance of the stream from the laboratory, the third method was most often employed.



FIGS. 1–13.*—Fig. 1, diagram of portion of plant showing relation of regenerated parts: *A*, before portion is cut; *B*, after cutting; *C*, after regeneration of organs has taken place. Fig. 2, sketch of portion of plant showing regenerated root. ×2½. Fig. 3, showing roots which have regenerated at both ends of original root fragment. ×3½. Fig. 4, portion of root which has regenerated two laterally concrescent roots; swellings represent paired shoots which have not yet emerged. ×3½. Fig. 5, stem fragment which has regenerated a shoot at its distal end and a root at its proximal end. ×3½. Fig. 6, camera lucida sketch of median longitudinal section of distal end of stem fragment showing location of meristem from which first two leaves have been initiated. ×22. Fig. 7, detailed drawing of meristem shown in fig. 6. ×150. Fig. 8, camera lucida sketch of longitudinal section of stem showing origin of third leaf. ×22. Fig. 9, same of longitudinal section of stem fragment showing origin of fourth leaf. ×22. Fig. 10, same of longitudinal section of stem fragment showing origin of fifth leaf. ×22. Fig. 11, sketch of young regenerated shoot showing form and position of first three leaves. ×5. Fig. 12, camera lucida sketch of transverse section of young regenerated shoot showing position of first three leaves (no vascular tissue in first leaf). ×33. Fig. 13, same of longitudinal section of stem fragment showing dichotomous branching of regenerated shoot. ×22.

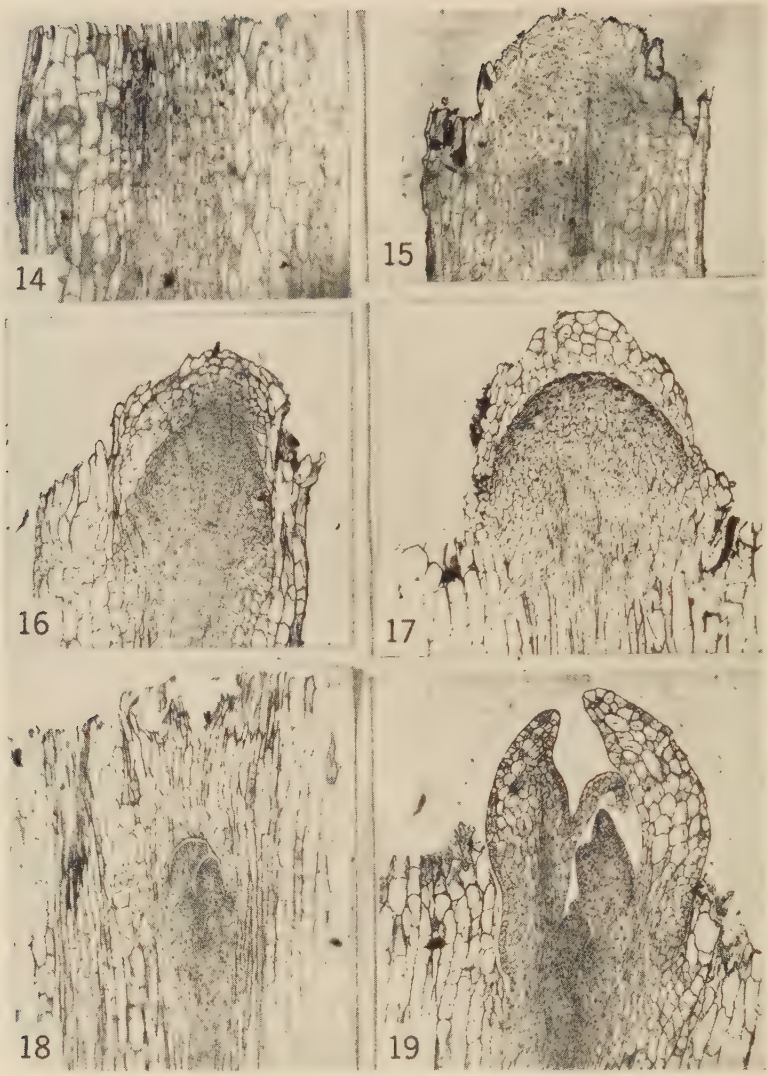
* Abbreviations: *dl*, dithecous leaf; *h*, hapteron; *l*, leaf; *r*, root; *rc*, root cap; *rh*, root hairs; *rm*, root meristem; *rr*, regenerated root; *rs*, regenerated shoot; *s*, stem; *sh*, shoot; *st*, intrapetiolar stipule; *vb*, vascular bundle.

It was found that root fragments produced roots only, while stem or leaf fragments produced both roots and shoots (fig. 1).

Regeneration from root

Pieces of root of various lengths were cut off and cultured. Samples for fixation and detailed examination were taken each day for ten or twelve days. Within four or five days a bulge appeared at the distal cut surface of each piece. This bulge was the beginning of the regenerated root which would have continued the axis of the original piece. Although a new root was always formed at the distal cut end (fig. 2), several cases of heteromorphism have been observed in which regeneration had also occurred at the proximal cut end (fig. 3), thus reversing the original polarity at this end of the piece.

The new root growing point is initiated just below the cut surface of the original root fragment. This is true of a fragment from any part of the parent root. The tissue of the central cylinder and the inner cells of the cortex about the central cylinder give rise to a meristem, which in turn gives rise to the growing point and root cap of the regenerated root. A longitudinal section of this region at such a stage shows all the cells of these tissues, except the tracheids, dividing by walls transverse to the root from the cut surface to a depth of about 0.5 mm. The earliest stages, however, show only scattered divisions (fig. 14). The cells of the new meristem continue to divide by transverse walls, and, by their subsequent growth and divisions, the meristematic mass begins to push out at the cut surface (fig. 15). In the meantime the apical growing region of the root is differentiated. This is formed at a distance of several cells from the cut surface (fig. 16). This region differs from that anterior to it by its smaller and more actively dividing cells. The cells of this anterior region are larger and less active than those beneath, and often include disintegrating tracheids of the original root. These anterior cells form the root cap (fig. 16). As growth of the regenerated root continues, the cells formed posterior to the growing point begin to elongate and soon the tissue layers of the body of the new root become differentiated. Its dorsal and ventral sides always correspond with those of the root which gives rise to it. The diameter of the root after it has emerged soon equals that of the original root, principally by the en-



FIGS. 14–19.—Fig. 14, longitudinal section through distal end of root fragment showing origin of meristem, which would have given rise to a new root growing point and root cap. ×60. Fig. 15, longitudinal section through distal end of root fragment showing origin of new root growing point and root cap. ×60. Fig. 16, sagittal section through distal end of root fragment; new root growing point and root cap clearly differentiated. ×60. Fig. 17, longitudinal section of proximal end of stem fragment showing origin of regenerated root. ×60. Fig. 18, longitudinal section of distal end of stem fragment showing origin of regenerated shoot. (This is a photograph of a similar section of same stage shown in fig. 8, except that the intrapetiolar stipule of the second leaf had not yet developed.) ×60. Fig. 19, photograph of section as shown in fig. 10. ×60.

largement of its cortical cells, thus leaving only a more or less inconspicuous collar of withering outer cells at the cut end of the old root.

Bifurcated roots are rather common in the field and often appeared in laboratory cultures (fig. 4). Although the exact mode of origin of such bifurcations was not ascertained, it is probably due to two root growing points instead of one originating at the broken end of a root; in fact two young regenerated roots are often found at the end of a broken root. It is then possible in cases of bifurcation that the tissue between two such growing points follows the growth of the latter, thus forming two roots which are laterally concrescent for a few millimeters or for a decimeter or more before the growing points become distinct (fig. 4). A transverse section of such a structure shows two cylinders. Four roots have occasionally been observed to diverge from an unusually broad root. Indications are that they are derived from two broken off, laterally concrescent roots from four fused growing points that arose at the broken end. In all observed cases of concrescence a collar is present basal to this, showing that regeneration of a broken root has taken place.

No shoots have been observed to arise at the cut or broken end of a root.

Regeneration of root from stem

The material found most satisfactory in this study was that of a large form growing at Difficult Run. The stems of this form are thicker (about 2 mm. in diameter) and the stem internodes are much longer than those of the ordinary forms, often attaining 15 mm. or more in length. Pieces of stems, 2–20 mm. or more long, were treated like the root pieces. Within four or five days a bulge appeared near the center of the proximal cut end, and a root soon appeared, always with its axis continuing that of the stem. Although out of its normal position with relation to the stem, the dorsiventral plane of the regenerated root corresponds to that of the stem. Before imbedding in paraffin, the basal end of each piece (about 5 mm. long) was cut off obliquely in such a manner that it could be properly oriented with respect to the plane of the bundles.

Within a day or two after the pieces were put in culture, the cells near the cut surface began to divide, usually by walls transverse to the axis of the stem. At first these divisions are scattered throughout

this part of the stem tissue, especially in and about the bundles. Then follows a period in which divisions occur in all planes, accompanied by a bulging at the cut surface, owing to the growth of the meristematic region beneath. This meristematic region extends from the cut surface to about 0.5 mm. or more below. Shortly after it begins to bulge, it becomes differentiated into two distinct regions, the outer one differing from the inner in that its cells are larger and the cytoplasm is less dense with smaller nuclei (fig. 17). This outer region, several cells in depth, develops to form a dorsiventral root cap. In the tissue of the meristematic region underlying this, the growing point of the root proper is initiated. Further growth of this root regenerated from a stem corresponds to that of one regenerated from a root.

Regeneration of shoot from stem

The stem fragments used were the same as those employed in the study of regeneration of a root from a stem, except that in this case the distal ends were studied. Soon after the cut surface of the distal end begins to bulge, the young leaves make their appearance (fig. 5). This is accompanied by the rupture of the tissue at the surface. Before imbedding in paraffin, just as in the case of the basal ends, these distal ends were cut diagonally for proper orientation, as the dorsiventrality of the young regenerated shoot also corresponds to that of the parent shoot.

The meristematic region which gives rise to the regenerated shoot is formed within one of the bundles at a distance of about 0.5–0.75 mm. from the distal cut surface. In this region, as may be seen in a longitudinal section, the elongated cells of the bundle begin, within a day or two after the cut is made, to divide by transverse walls. They continue to divide in the same plane until definite longitudinal rows of small isodiametric cells are formed. At first the small cells of these longitudinal rows have approximately the same transverse diameters as those of the original rows. Soon cells near the center of this meristematic region begin to enlarge and become distinguished from the others by their larger nuclei and denser cytoplasm. These cells, still arranged in longitudinal rows, form two groups, each having the general shape of a young leaf, and together they suggest the pair of cotyledons of a dicotyledonous seedling (figs. 6, 7). The shape

of each of the first two leaves of the regenerated shoot soon becomes distinct; that is, each becomes sharply delimited, except at its base, from the surrounding stem tissue. The two are usually unequal in size, and often one arises before the other. The plane joining these two leaves always corresponds to that passing through the two ranks of leaves of the original shoot. In the meantime the elongated cells of the remainder of the bundle, above the primary leaves, divide by transverse walls (fig. 7). These divisions occur progressively from the young meristematic region toward the distal cut surface of the stem.

From these first two leaves the regenerated shoot arises. There is no stem growing point. Once the leaves are fully initiated within the meristematic region, they begin to enlarge by the growth and the division of their cells in all planes. Each leaf becomes surrounded by a distinct layer of cells, the epidermis, and each pushes upward and in so doing ruptures the tissue surrounding it except at its base. The first leaf is easily distinguished by its larger size (fig. 7) and by the character of its subsequent development. On the inner surface near its base a protuberance nearly half the length of the leaf itself is then initiated by the growth and divisions of the cells beneath the epidermis, accompanied by growth and anticlinal divisions of the cells of the latter. This protuberance is the rudiment of the third leaf (figs. 8, 18). On the inner surface of the second leaf, just above the third leaf rudiment, there is formed the rudiment of the intrapetiolar stipule of the second leaf (fig. 8). The portion of this leaf above the rudiment of the intrapetiolar stipule develops to form its blade. By further growth the intrapetiolar stipule of this second leaf comes to inclose the rudiment of the third leaf. As growth proceeds, the rudiment of the fourth leaf is initiated at the base of the second leaf (fig. 9). At this time the rudiment of the third leaf begins to differentiate into a blade and intrapetiolar stipule. This stipule incloses the rudiment of the fourth leaf just as that of the second leaf incloses the rudiment of the third leaf. The fifth leaf (figs. 10, 19) and all subsequent leaves arise in the same manner as the third and fourth leaves, each being produced from the base of the *second* older leaf. The intrapetiolar stipule of any given leaf is initiated just after the appearance of the rudiment of the next younger leaf and at first it always incloses this rudiment.

By the time the fourth leaf rudiment has arisen, the outer tissues at the cut surface of the stem are usually ruptured and the young regenerated shoot has emerged. The first two leaves, which, as already mentioned, differ from the subsequent ones in that they arise from dedifferentiated cells of the original stem tissue, remain rather small, and differ in form from the subsequent leaves. The first leaf in transverse section is crescent-shaped (fig. 12). At an early stage in its history, its apex and lateral margins, by continued growth, come to inclose the second leaf (and later leaf rudiments) except at its dorsal side (fig. 8). The first leaf continues to grow at relatively the same rate as the second leaf and later leaf rudiments, until shortly after the young shoot has emerged, when its growth ceases. The first leaf rarely develops a blade, and it may be considered equivalent to the base only of one of the later leaves. The second leaf, although it develops a blade, as do the subsequent ones, differs from the latter in that its blade, which continues to grow for a short time, does not become differentiated into forked linear lobes. The third and all later leaves, however, do show such a differentiation (fig. 11). Hence the second leaf is a structure "transitional" between the first and third leaves.

The axis of the regenerated shoot always continues that of the original shoot and its plane of symmetry is also the same as that of the latter. The growth of the young shoot after it has emerged is rapid. Its stem, which continues to increase in radial diameter, soon attains that of the original stem and the juncture between the two becomes more and more difficult to distinguish, until finally there is left only a narrow ring or collar to indicate that regeneration has occurred.

As already stated, there is no stem growing point and each leaf arises from the base of the *second* older leaf. Therefore, the elongation of the stem axis is brought about by the growth and divisions of cells principally at the bases of the newly formed leaves. The vascular system of the young regenerated shoot begins differentiation by the time the third or fourth leaf rudiment has arisen. Within the central region of the meristematic tissue beneath the young leaf bases, a group (sometimes two or more groups) of tracheids is formed from the elongated cells of this region. These tracheids arise inde-

pendently of the original stem tracheids, which by this time have usually become disintegrated. The new tracheids continue to differentiate to a considerable distance within the bundle, toward the base of the stem. The cells within the young leaves, except those of the first, soon begin to elongate; and into the leaves bundles of tracheids extend from the meristematic tissue beneath the young leaf bases. There is neither a trace of a bundle in the first leaf (fig. 12) nor do tracheids extend in that direction from below. From the group of tracheids in the meristematic tissue, a bundle of tracheids extends into the second and third leaves at about the same time. The bundle of the second leaf, however, often arises independently of the group of tracheids. Shortly after the fourth leaf rudiment appears, the bundle which extends into it arises from that of the third leaf. The bundle of the fifth leaf, after it is formed, branches from that of the fourth. This method of bundle formation is the same in all subsequent leaves, so that a transverse section of the stem would show usually three bundles arranged in a crescent-shaped row, of which the concave side faces the dorsal side of the stem (the side toward the root).

When the rows of tracheids first appear in the bundle of the young leaf, they may at times extend at least half the length of the young leaf. But as growth proceeds, the further formation of tracheids gradually ceases. Consequently any transverse sections of a mature leaf, except at the base of the petiole, would not exhibit xylem elements. And even here, owing to an extensive stretching by the rapidly growing surrounding tissues, the only indications of their having been present may often be annular or spiral thickenings of those portions of the cells which have collapsed, accompanied by the pressing inward of the adjacent parenchyma cells of the thin parts of the walls between the rings or spiral thickenings. This stretching of the xylem elements also may occur in the rapidly growing region of the stem below the leaf bases, and frequently no xylem elements can be detected.

Branching of the young shoot frequently occurs and may take place when the young leaf rudiments are being formed at the time of regeneration (fig. 13). There are some indications that branching at this early stage is associated with the branching of the bundles of

the original stem. In such cases the first and second leaves appear to arise in the usual manner, and at the base of the first leaf the rudiment of the third leaf arises just as in the ordinary method of regeneration. Simultaneously with initiation of the first leaf or sometimes later, a leaf, equivalent to the first, is differentiated from the meristematic tissue at the side of the second leaf opposite the first in such a manner that the second leaf stands between and in the same plane with the two equivalent leaves. A third leaf arises at the base of each of the equivalent ones and each third leaf is inclosed at first by the intrapetiolar stipule of the second leaf (which is a dithecous one as shown at *l2* (fig. 13, *dl*). This gives rise to a type of dichotomy, and further growth of the two young shoots is similar to that of the ordinary branched shoots in this plant.

Regeneration from leaf

The leaf also shows a marked capacity for regeneration (fig. 1). When leaves are broken off from plants in their natural habitat, the detached parts are washed away and consequently their capacity to regenerate is easily overlooked. However, the laboratory method of culture shows that leaves can regenerate if cut off close to the stem. If they are cut off too far from the stem, they usually die.

A detached leaf produces at its proximal cut end a root, which continues the axis of the leaf. This root arises from the vascular bundle of the leaf in the same manner that a regenerated root arises at the basal cut end of the stem. On the other hand, if the cut is such as to leave part of the petiole (0.5 mm. or less) on the stem, a shoot often arises from the distal end of this. If the leaf is removed entirely, regeneration occurs from the stem at the point of detachment. A shoot, which continues the axis of the leaf, arises from the leaf bundle at the distal cut end in much the same manner that a shoot arises from a vascular bundle at the distal cut end of a stem.

Summary

1. *Podostemon ceratophyllum* has an unusual capacity for regeneration, which is possessed by each of its vegetative organs, and this regeneration can readily be observed in laboratory cultures.

2. If the distal end of a root is cut off, a new root growing point is

initiated back of the cut surface. The root cap is formed from the tissue between the meristem of the root growing point and the cut surface.

3. Occasionally cases of heteromorphism occur in root cuttings.

4. In contrast to known cases of regeneration of root tips in other forms, a new root growing point may be formed in *Podostemon ceratophyllum* regardless of how much of the distal end of the original root is cut off.

5. A root is also initiated at the proximal cut end of a stem, and it continues the axis of the latter. Its dorsal and ventral sides correspond to those of the stem.

6. A shoot is regenerated at the distal cut end of a stem. The first two leaves of the regenerated shoot arise from dedifferentiated cells within the stem bundle a short distance back of the cut surface. The regenerated shoot continues the axis and the dorsiventral symmetry of the original stem.

7. A detached leaf regenerates a root at its proximal end; the distal end of the petiole regenerates a shoot. In both cases the regenerated organ continues in line with the axis of the original organ.

8. When two or four roots are regenerated at the same cut end of a root, these may be laterally concrescent for 1 mm. to 10 cm. or more. Two shoot axes also may arise simultaneously at the distal end of a stem fragment. These are not concrescent.

The writer wishes to acknowledge indebtedness to Professor Duncan S. Johnson, under whose direction this investigation was carried out, for his helpful suggestions and valuable criticisms.

Johns Hopkins University
Baltimore, Maryland

LITERATURE CITED

1. Hammond, B. L., Development of *Podostemon ceratophyllum* Michx. Bull. Torr. Bot. Club. In press. 1936.
2. Warming, E., Familien Podostemaceae. Kongel. Dansk. Videnskab. Selskabs Skrifter. Sjette Raekke. 1:1–34. 1881.
3. Went, F. A. F. C., Morphological and histological peculiarities of the Podostemonaceae. Proc. Internat. Congress Plant Sci., Ithaca. 1:351–358. 1926.

VITA

Bayard Louis Hammond was born in Elkland, Pennsylvania, March 26, 1900. There, he received his elementary, secondary, and high school education. In 1918 he entered Mansfield State Normal School, where he received a teacher's certificate in 1920. During the scholastic year of 1920-21, he was principal of Osceola High School, Osceola, Pennsylvania. The following four years (1921-25), he taught in the public schools of Puerto Rico. In September, 1925, he was appointed acting head of the Spanish Department of Lebanon Valley College, where he also pursued studies in botany, was assistant in botany (1927-29), and was awarded the degree of Bachelor of Arts in 1929. In October, 1929, he entered The Johns Hopkins University as a graduate-student assistant in the Department of Botany, a position which he held until June, 1933. During the years 1931-34, he was part-time technical assistant to Dr. L. M. Langdon of Goucher College. During the years 1932-34, he was also part-time technical assistant to Dr. Ralph E. Cleland of the same institution, and to whom he was full-time research assistant in the cytogenetics of Oenothera during the year 1934-35.